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(54) MEGSIN ACTIVITY INHIBITOR

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a polypeptide inhibiting the activity of Megsin and to provide an application of the same.
SOLUTION: This invention provides the polypeptide inhibiting the activity of Megsin and a composition for therapy and/or prevention of mesangial proliferative glomerulonephritis that contains the polypeptide inhibiting the activity of Megsin as an active component. The polypeptide inhibiting the activity of Megsin is useful for therapy of prevention of mesangial proliferative glomerulonephritis because the Megsin induces the mesangial proliferative glomerulonephritis.

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CLAIMS

[Claim(s)]
[Claim 1] The polypeptide which checks the Meg Singh activity.
[Claim 2] Array number: The polypeptide according to claim 1 constituted with the peptide which has the amino acid sequence indicated by 1.
[Claim 3] The kidney disease therapy and/or preventive which make an active principle a polypeptide according to claim 1 or 2.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the constituent for the therapy of the constituent for the therapy of the mesangial proliferative glomerulonephritis using the polypeptide and this polypeptide which constitute a specific cel pin (serine protease inhibitor), i.e., Meg Singh's (Megsin) reactant loop-formation field, and/or prevention, and/or prevention.

[0002]
[Description of the Prior Art] Renal failure is symptoms in which a kidney disease patient results eventually. The cause or experience are not uniform, and also when [many] the symptoms of renal dysfunction develop and it results in renal failure by lesions other than the original kidneys, such as drug intoxication, an infectious disease, a malignant tumor, diabetes mellitus, and systemic lupus erythematosus (SLE), it sees.
[0003] In the terminal renal failure on which the blood filtration or the detoxifying effect of the kidney do not function at all, although the renal transplantation is the only therapy means, in our country, it is hard to say that the supply organization of kidney graft is fully improved. Moreover, the social cognition to the transplantation therapy itself is not progressing, either. It does not pass over the example of the renal transplantation of our country in about [per year] 700 case, and this numeric value has not carried out the increment in the number year of here. Therefore, the actual condition is that dialysis is the only cure as a kidney substitution therapy.
[0004] Now, the terminal renal failure dialysis patient of our country counts about 210,000 presumption, and is a world primacy in the number of patients per population. The per capita average cost of medical treatment needs every year in about 6 million yen, and is set to one of the big causes which suppress a health care insurance system. moreover, a patient since 4 - 6

hours will be restrained for one day for two to three days every week for dialysis treatment -- a principal's social rehabilitation is also difficult.
[0005] Furthermore, dialysis patient age is also going up with aging of population in recent years. For this reason, kidney disease is treated at an early stage, and the need for the drugs which prevent progress to renal failure is recognized. However, a kidney disease field is deficient in information research bases, such as a target molecule for an innovative drug development, and the actual condition is that effective drugs are not born.
[0006] the organ as which a mesangial cell is not regarded except the kidney -- it is a specific cell and bearing the structure of mesangium and a role important for functional maintenance is known well. Moreover, since the increment in the extra-cellular matrix secreted from the own growth and own MESANGISAMU cell of a mesangial cell etc. is accepted at the time of a glomerulus failure, it is surmised that it is the cell which participates also in the onset and progress of a disease deeply. In order to solve the molecule mechanism of a glomerulus failure from these things, it is considered to be indispensable to solve the biochemical character of a mesangial cell first. However, the singularity of the gene level about a mesangial cell was not clarified.
[0007] Although about 60 trillion cells exist in human the living body and these have the same genomic DNA, it is thought that having the biological property from which each cell, as a result the organ differed is based on the gene specifically discovered to each cell or an organ. this invention persons thought it possible to detect a specific high manifestation gene cluster to a mesangial cell, when clarifying the profile of the gene cluster discovered to a mesangial cell. And the gene cluster which participates in the condition of glomerulonephritis from the inside could also be determined, the clue which solves the molecule mechanism of a glomerulus failure was also found, and it was considered that the development of the cure for new glomerulonephritis based on it also became possible.
[0008] Then, this invention persons clarified the gene expression pattern of a mesangial cell, and tried to analyze the cell property on gene level. First, this invention persons extracted mRNA from the culture Homo sapiens mesangial cell for the purpose of analyzing quantitatively the gene discovered to a mesangial cell, and produced the 3'-directedcDNA library. And [Yasuda which enforced large-scale DNA sequencing and database analysis of the gene fragment inserted in the clone, Y.et al.:Kidney Int., 53:154-158, 1998].
[0009] Consequently, the gene which consists of overall-length 2,249bp which named it Meg Singh as a gene especially discovered strongly by the mesangial cell was isolated. And it succeeded in isolating and acquiring the Meg Singh protein which is new protein with which Meg Singh's overall-length cDNA clone consists of 380 amino acid which carries out a code.

Furthermore, amino acid homology retrieval by the FASTA program was performed using the SwissProt amino acid sequence database. and In the amino acid sequence of Meg Singh protein, serine protease inhibitor (cel pin: SERPIN) super family [Carrell, R.W.et al.:Trends Biochem.Sci., 10:20, 1985;Carrell, and R.W. et al.:Cold Spring Harbor Symp.Quant.Biol., 52:527, 1987;Kruithof, E.KO.et al.:Blood, and 86, 4007 and 1995 ;P otempa, J.etal.:J.Biol.Chem., and 269: -- 15957 and 1994; It is supposed that it is at least as the bioactive core of Remold-O'Donnell, E.FEBS Lett., 315:105, and 1993]. It found out that the similar array (EEGTEATAAT / array number: 3) existed in the consensus sequence (EEGTEAAAAT / array number: 2) in an important reactant loop-formation field (reactive loop site). That is, Meg Singh is [Miyata and T.et al.:J.Clin.Invest. in which it has the structural description of a cel pin and the reactant loop-formation field (P17-P5':EEGTEATAATGSNIVEKQLPQS / array number: 4) which is an active site like other cel pins exists, 102:828-836, and 1998]. [Miyata which made it clearer than these things that HITOMEGUSHIN protein was protein belonging to a cel pin, T.et al.:J.Clin.Invest., 120:828-836, 1998]. And patent application of these knowledge was carried out (WO 99/15652).

[0010] Antithrombin, plasminogen activator inhibitor (plasminogen activator inhibitor:PAI), ovoalbumin, etc. are known by the cel pin. These cel pin molecules have high homology of each other, and are [the three-dimensional structure is also very good and] alike. A cel pin forms the target serine protease and complex, respectively, and checking enzyme activity is known. It is the flexible reactant loop-formation field (reactive loop site) of a cel pin which is combined with the active site of a protease.

[0011] Besides the condition of having been cut by the cel pin with the active state, a polypeptide chain is inactive completely but functionally, and has the ****-condition of saying that it does not combine with a protease. In current, about these [of a cel pin] three kinds of all conditions By X-ray crystallographic analysis [Huber and R. as which structure is determined et al.: Biochem., 28: 8951, 1989;Baumann, and U. et al.: J.Mol.Biol., 218:595, 1991;Schulze, A.J.et al.:Biochem., 31:7560, 1992;Goldsmith, E.J.et al. :P roteins Struct.Funct.Genet., 9:225, 1991;Goldsmith, E.J.etal.:Nature (London), 355:270, 1992;Schreuder, H.et al.:J.Mol.Biol., 229:249, 1993].

[0012] Cel pin mold folding consists of dense structure which three antiparallelism beta sheets A, B, and C selectively covered by the alpha helix make. It is thought that the structure of the ovoalbumin which is not cut is the criteria form of a cel pin, and Sheet A contains five strands. The strand (beta 16) which a flexible loop formation begins from the edge of the strand 5 of the beta sheet A, an alpha helix is in the outside of a molecule continuously, and is in the edge of the beta sheet C further continues, and the start point of the strand (beta 17) contained in beta sheet is reached.

There is a part where a cel pin is divided by the alpha-helix field of the center of a loop formation, and it has projected here like a handle on the outside of a molecule.

[0013] Among beta5 and beta15 of the beta sheet A, although the flexible loop-formation field of active antithrombin is in a general position as well as ovoalbumin, some residue near the beginning of a loop formation forms 6th short beta strand. Moreover, there is no alpha helix in the loop formation which has come out of a part for the body of a molecule, and it can insert in the active site of a thrombin as it is. That is, by the cel pin of an active state, the loop-formation field serves as a form projected from a part for the body of a cel pin molecule so that it can combine with the active site of a serine protease (drawing 1 (a)).

[0014] In the cutting die of alpha 1-antitrypsin, the one half of the about [the cutting section] from a loop-formation start of region forms perfect beta strand, and it is inserted among the strands beta5 and beta15 of the beta sheet A. That is, a cel pin molecule is dissociated depending on the case, although a protease and noncovalent bond complex (Michaelis complex) are once formed. A cel pin molecule is then cut by the lobe of the loop-formation field which is an active site. By the cel pin of a cutting condition, the amino terminal section of a loop-formation field enters among the beta strands 5 and 15, and forms long beta strand in the center of beta sheet (drawing 1 (b)). The second half section of a loop-formation field occupies the almost same location as the case of active antithrombin. There are two new ends (connected in the active type) produced by fragmentation in the ends of a molecule, respectively, and it is separated from them about 70A.

[0015] Finally, although beta strand has joined the beta sheet A as well as the cutting die of alpha 1-antitrypsin in the state of **** of PAI, the remaining part of a flexible loop-formation field constituted the loop formation from the exterior of a molecule, and is connected with beta strand of the beta sheet B, and there is no strand of the edge of the beta sheet C. In the state of this **** that is the most stable and does not have activity, the form of beta strand where the amino terminal part of a loop-formation field was inserted into beta sheet as well as the condition of having been cut is taken. The remaining residue is [(drawing 1 (c)) Carrell, R.W.et al.:Structure, 2:257-270, and 1994] which form a loop formation at the edge of the opposite hand of beta sheet. namely, [which the loop-formation field which is the active site of a cel pin takes three kinds of gestalten, an "active type", "the cut condition", and a "**** condition", and depends also for activity on the gestalt -- Yamasaki, M.et al.:J.Med.Biol., and 315:113- 120, 2002, Saunders.D.N.et al.:J.Biol.Chem., 276:43383-43389, and 2001].

[0016] If the changeover from an active type to a **** type takes place, a

loop formation will change to long beta strand, and will be inserted in the center of beta sheet. In order to cause a big structural change which inserts beta strand in stable beta sheet already constituted, the strand with which each other was adjoined in beta sheet must separate first. In order to take dense structure, this not only rearranges canal-contact of a large number built inside the molecule, but means that much hydrogen bond goes out. The contact for the hydrogen bond newly formed or restoration must be rebuilt after excessive beta strand is inserted. Before the structure determination of a cel pin, anticipation was not carried out at all, either, and such a big change that takes place to beta structure is not observed yet by any systems other than this [Branden, C.et al.:Introduction to Protein Structure Second Edition, 1999]. By the protease, the inside of a loop formation (P1-P1' part) is cut, and an active cel pin molecule once forms noncovalent bond complex (Michaelis complex) (drawing 2 A-> drawing 2 B). Then, the amino terminal of a loop-formation field enters between the beta sheets A, complex serves as covalent bond, and a protease is inactivated (drawing 2 C). However, by the case, non-sharing complex is dissociated, the condition (inactive mold (drawing 1 B)) and protease which were cut are not influenced by the cel pin molecule by the cel pin, but an active type is maintained.

[0017]

[Problem(s) to be Solved by the Invention] The object of this invention is offering the inhibitor of the Meg Singh activity effective in the therapy of the disease relevant to a mesangial cell etc. Moreover, this invention relates to the constituent for the therapy of the constituent for the therapy of the mesangial proliferative glomerulonephritis using the polypeptide and this polypeptide which constitute Meg Singh's reactant loop-formation field, and/or prevention, and/or prevention.

[0018]

[Means for Solving the Problem] Meg Singh has the structural description of a serine protease inhibitor (SERPIN), and the reactant loop-formation field (P17-P5':EEGTEATAATGSNIVEKQLPQS / array number: 4) which is an active site like other cel pins exists (Miyata, T.et al.:J.Clin.Invest., and [102:828, 1998]). On the other hand, since Meg Singh's transgenic mouse shows amplification of a progressive mesangium substrate, the increment in the cell in a glomerulus, and the increment in an immune complex deposit, possibility that a nephritis is caused with the cel pin activity is suggested strongly. [585 Miyata, T.et al.:J.Clin.Invest., 109, 2002].

[0019] Since Meg Singh is a glomerulus high manifestation gene belonging to a cel pin super family, this gene is considered to have relevance in the onset/progress of glomerulonephritis with the functional gene in a glomerulus. And since it has the reactive loopsite structure where Meg Singh protein is peculiar to the subgroup which has protease activity inhibition

ability also in a cel pin super family, it is possible to work as repressor to a certain kind of serine protease.

[0020] Protease activity is kept constant by the balance of the activity between the repressors to a protease and it, and it is known that the fluctuation will affect the function and symptoms of an organization/cell remarkably. in fact, the resolution according to the protease of extra-cellular-matrix protein in the time of glomerulosclerosis -- lowering is seen. Moreover, although it compares with a wildness mouse and a bleomycin reactivity fibrosing disease is induced by high rate as another example with the high manifestation mouse of PAI-1 (the amino acid homology with Meg Singh protein is 27.7%) which is a kind of a cel pin, the research result that a fibrosing disease cannot happen easily is also reported to reverse by the PAI-1 deficit mouse (Eitsman, D.T.et al.:J.Clin.Invest., and [97:232, 1996]).

[0021] Furthermore in recent years a cel pin not only controls serine protease activity, but It participates also in blood coagulation, the fibrinolysis, an inflammatory response, cell differentiation/growth, and apotosis. [Kruithof to which it is reported that the physiology device and pathophysiology-meaning are various, E.KO.et al.:Blood, 86, I:4007, 1995;Bachmann, F.Thromb.Haemostasis, 74: 172, 1995;Tsujimoto, and M. et al.:J.Biol.Chem. and 272:15373, 1997;Bird, P.I.Results Probl.Cell.Differ., and 24: -- 63 and 1998 ;P otempa, J.et al.:J.Biol.Chem., 269:15957, and 1994].

[0022] Furthermore, when various organizations and cells were analyzed by the Northern blot and reverse transcription polymerase chain reaction, Meg Singh had the weak manifestation in Homo sapiens fibrocyte, a smooth muscle cell, an endothelial cell, and keratinocyte, and it turned out that it is strongly discovered especially by the mesangial cell. That is, the Meg Singh gene expression has singularity in a mesangial cell. These knowledge is in situ hybridization further. [Miyata, T.et al.:J.Clin.Invest., 102:828, 1998;Suzuki, D.et al.:J.Am.Soc.Nephrol., 10:2606, and 1999] And immunohistochemical method using the Meg Singh antibody [Inagi, R.et al.:Biochem.Biophys.Res.Commun., 286:1098, and 2001] It was checked.

[0023] Moreover, if a IgA glomerulonephritis patient, a diabetic nephropathy patient, and healthy people compare the amount of manifestations of Meg Singh under kidney tissue In a IgA glomerulonephritis patient or a diabetic nephropathy patient There are many amounts of manifestations of Meg Singh intentionally. [Miyata and T. et al.: J. Clin.Invest., 102: 828, 1998;Suzuki, D.et al.:J.Am.Soc.Nephrol., and 10: -- 2606, 1999, Inagi, R.et al.:Biochem.Biophys.Res.Commun., 286:1098-1106, and 2001]. Moreover, in the experimental mesangial-proliferative-glomerulonephritis model (Thy-1 nephritis model) using Latt, lifting of the same amount of Meg Singh manifestations was accepted. [60:641 Nangaku, M.et al.:Kidney Int., 2001]. Meg Singh's manifestation changed from this in connection with the

malfunction of a mesangial cell, and it became clear to participate in the onset/progress of a disease deeply.
[0024] In order to understand Meg Singh's role in the function of the mesangium further, we did the superfluous manifestation of the cDNA of HITOMEGUSHIN by the mouse genome. Two Meg Singh TRANS GENIC mice were obtained, and they showed amplification of a progressive mesangium substrate, growth of a mesangial cell, and the increment in an immune complex deposit [Miyata, T.etal.:J.Clin.Invest., 109, 585 and 2002, WO 01/24628]. These knowledge shows that Meg Singh has important effect on the function of the mesangium biologically. An interesting thing can be made to generate that Meg Singh's single genetic manipulation is experimental, and the first stage mesangium lesion which exists in Homo sapiens glomerulonephritis. Thus, also in the animal individual, it is reported that Meg Singh participates in the onset of mesangial proliferative glomerulonephritis.
[0025] Therefore, a header and specifying are effective in the therapy of the disease relevant to investigation of the cause of the disease relevant to a break through of the biological property of a mesangial cell, and a mesangial cell in the matter which has the activity which can check the activity to Meg Singh, and the polypeptides which were especially excellent in singularity, as a result a mesangial cell, a diagnosis, etc.
[0026] this invention persons completed a header and this invention for the ability of the polypeptide which has the specific array which consists of TEATAATGSNIVEK (array number: 1) equivalent to P1-P14 array in Meg Singh's reactant loop-formation field to check the Meg Singh activity, as a result of searching for the polypeptide which has inhibitory action for recombination Meg Singh's plasmin activity inhibition ability to the Meg Singh activity against an index. Although the array of Meg Singh's reactant loop-formation field (P17-P5':EEGTEATAATGSNIVEKQLPQS / array number: 4) was known, knowledge that the polypeptide of the specific part in a reactant loop-formation field checks the Meg Singh activity was not checked. The polypeptide by this invention can check a reaction with the plasmin which is a serine protease to Meg Singh and Meg Singh. That is, this invention relates to the following polypeptide and its application.
[1] The polypeptide which checks the Meg Singh activity.
[2] Array number : a polypeptide given in [1] constituted with the peptide which has the amino acid sequence indicated by 1.
The kidney disease therapy and/or preventive which make the polypeptide of a publication an active principle [3], [1], or [2].
[0027]
[Embodiment of the Invention] This invention offers the polypeptide which checks Meg Singh's activity. In this invention, inhibition of Meg Singh's activity means that Meg Singh's cel pin activity falls. Moreover, cel pin

activity is the inhibitory action to the protease activity of a serine protease. In this invention, not only perfect control of cel pin activity but partial control is included with inhibition.

[0028] It can check in the example 2 that a polypeptide checks Meg Singh's activity by the approach of a publication. Namely, the inhibition activity can be checked by making depressor effect of a polypeptide over Meg Singh's protease activity inhibition ability into an index, using plasmin as a serine protease to Meg Singh. Protease activity can be measured by observing digestion of for example, a fluorescence substrate.

[0029] It found out that the polypeptide from which this invention persons constitute Meg Singh's reactant loop-formation field had the inhibitory action of the Meg Singh activity. Especially the polypeptide that constitutes Meg Singh's reactant loop-formation field means the field set to array number:1 from the amino acid sequence of a publication among Meg Singh's amino acid sequence. Therefore, the polypeptide of this invention is a polypeptide set to array number:1 from the amino acid sequence of a publication.

[0030] The polypeptide by this invention contains the polypeptide set to array number:1 from the amino acid of a publication as an active principle. the polypeptide of this invention -- laws, such as a chemical synthesis method, -- it can obtain by the method. Moreover, various processings of diluting in remaining as it is or water can be performed and used for the remedy constituent for treating and/or preventing the mesangial cell fecundity nephropathy containing the polypeptide of this invention, and it can blend and use them for drugs, quasi drugs, etc. Although the loadings in this case are suitably chosen according to symptoms or a product, since properties, such as the stability of the product itself and a flavor, may be spoiled if it can consider as 0.01 - 10 % of the weight, and the prevention or the therapy operation which will be satisfied if fewer than 0.001 % of the weight may not usually be accepted 0.001 to 50% of the weight especially in the case of whole body administration pharmaceutical preparation and 5 % of the weight is exceeded, they are not desirable.

[0031] The polypeptide of this invention may be contained in pharmaceutical preparation as a salt permitted in galenical pharmacy. As a salt permitted in pharmaceutics, acid addition salts, such as a salt with bases, such as an inorganic base and an organic base, an inorganic acid, an organic acid, basicity, or acidic amino acid, etc. are mentioned, for example. As an inorganic base, alkaline earth metal, such as alkali metal, such as sodium and a potassium, calcium, and magnesium, aluminum, ammonium, etc. are mentioned, for example. As an organic base, tertiary amines, such as secondary amines, such as primary-amines [, such as ethanolamine,], diethylamine, diethanolamine, dicyclohexylamine, N, and N'-dibenzyl ethylenediamine, a trimethylamine, triethylamine, a pyridine, picoline, and

triethanolamine, etc. are mentioned, for example. As an inorganic acid, a hydrochloric acid, a hydrobromic acid, a nitric acid, a sulfuric acid, a phosphoric acid, etc. are mentioned, for example. As an organic acid, a formic acid, an acetic acid, a lactic acid, trifluoroacetic acid, boletic acid, oxalic acid, a tartaric acid, a maleic acid, a benzoic acid, a citric acid, a succinic acid, a malic acid, methansulfonic acid, ethane sulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, etc. are mentioned, for example. As a basic amino acid, an arginine, a lysine, an ornithine, etc. are mentioned, for example. As acidic amino acid, an aspartic acid, glutamic acid, etc. are mentioned, for example.

[0032] As a medication method of the remedy constituent of this invention, in addition to internal use and intravenous administration, permucosal administration, dermal administration, intramuscular administration, hypodermic administration, intrarectal administration, etc. can choose suitably, and it can use as various pharmaceutical preparation according to the medication method. Although each pharmaceutical preparation is indicated below, the pharmaceutical form used in this invention is not limited to these, and can be used as various pharmaceutical preparation usually used in the remedy pharmaceutical preparation field.

[0033] When using for the therapy to <whole body administration pharmaceutical preparation> mesangial proliferative glomerulonephritis, and/or prevention, the amount of internal use of a polypeptide has the desirable range of 0.003 mg/kg - 3 mg/kg, and it is 0.03 mg/kg - 0.3 mg/kg more preferably. although there is fluctuation with young and old of both sexes or a form especially in intravenous administration when performing whole body administration -- effective blood drug concentration -- 0.2microg/mL - 20microg/mL -- a medicine should be prescribed for the patient so that it may become the range of 0.5microg/mL - 10microg/mL more preferably.

[0034] As a pharmaceutical form in the case of administering orally, there are powder, a granule, a capsule, a pill, a tablet, elixirs, suspension, an emulsion, syrups, etc., and it can choose suitably. Moreover, gradual-release-izing, stabilization, ******-izing, formation of difficulty breaking, enteric-izing, easy absorption-ization, etc. can be embellished about these pharmaceutical preparation. Moreover, as a pharmaceutical form in the case of performing oral cavity internal division place administration, there are a peptizing agent, a hypoglottis agent, a buccals, trochiscus, an ointment, a application-with-gauze agent, liquids and solutions, etc., and it can choose suitably. Moreover, gradual-release-izing, stabilization, ******-izing, formation of difficulty breaking, enteric-izing, easy absorption-ization, etc. can be embellished about these pharmaceutical preparation.

[0035] About each above-mentioned pharmaceutical form, the technique of a

well-known drug delivery system (DDS) is employable. The DDS pharmaceutical preparation told to this description means pharmaceutical preparation made into the optimal formulation after taking into consideration a route of administration, bioavailability, a side effect, etc., such as gradual release-ized pharmaceutical preparation, topical application pharmaceutical preparation (troches, the buccal tablet, sublingual tablet, etc.), drug release control pharmaceutical preparation, an enteric coated preparation, and stomach solubility pharmaceutical preparation.

[0036] The short drug of the half-life when blood drug concentration falls promptly [when it consists in the component of DDS of a drug, a drug release module, an encapsulation object, and a therapy program fundamentally and especially bleedoff is stopped about each component] is desirable, the body tissue of an administration part and the encapsulation object which does not react are desirable, and it is desirable to have the therapy program which maintains the best drug concentration in the set-up period further. The drug release module has a drug storage shed, the bleedoff control section, the energy source and the bleedoff hole, or the bleedoff front face fundamentally. All of these elemental ingredients do not need to gather, they can perform addition or deletion suitably, and can choose the best gestalt.

[0037] There are a giant molecule, a cyclodextrin derivative, lecithin, etc. as an ingredient which can be used for DDS. a giant molecule -- an insoluble giant molecule (silicone and an ethylene-vinylacetate copolymer --) An ethylene-vinylalcohol copolymer, ethyl cellulose, cellulose acetate, etc., a water soluble polymer and a hydroxyl gel formation macromolecule (polyacrylamide --) A polyhydroxyethyl methacrylate bridge formation object, the poly acrylic bridge formation object, Polyvinyl alcohol, polyethylene oxide, a water-soluble cellulosic, sustained-soluble polymer (ethyl cellulose --), such as a bridge formation POROKI summer, a chitin, and chitosan The partial ester of the methyl vinyl ether and a maleic-anhydride copolymer etc., a stomach solubility giant molecule (the hydroxypropyl methylcellulose and hydroxypropylcellulose --) Carmellose sodium, macro gall, a polyvinyl pyrrolidone, a methacrylic acid dimethylaminoethyl methacrylic acid methyl copolymer, etc., an enteric giant molecule (hydroxypropylmethylcellulose phthalate and an acetic-acid FUTARU cellulose --) Hydroxypropyl-methylcellulose acetate succinate, carboxy methyl ethyl cellulose, biodegradable polymers (thermal coagulation or bridge formation albumin --), such as an acrylic-acid system polymer There are bridge formation gelatin, a collagen, a fibrin, poly cyanoacrylate, polyglycolic acid, polylactic acid, Pori beta hydroxyacetic acid, a poly caprolactone, etc., and it can choose suitably by the pharmaceutical form.

[0038] Especially the partial ester of silicon, an ethylene-vinylacetate

copolymer, an ethylene-vinylalcohol copolymer, and the methyl vinyl ether and a maleic-anhydride copolymer is applicable to bleedoff control of a drug, cellulose acetate can be used as an ingredient of an osmotic-pressure pump, ethyl cellulose, the hydroxypropyl methylcellulose, hydroxypropylcellulose, and methyl cellulose can be used as a film raw material of a sustained release drug, and the poly acrylic bridge formation object can be used as the tunica mucosa oris or an eye membrane adhesion agent.

[0039] Moreover, in pharmaceutical preparation, additives, such as a solvent, an excipient, a coating agent, a basis, a binder, lubricant, disintegrator, a solubilizing agent, a suspending agent, a viscous agent, an emulsifier, a stabilizer, a buffer, an isotonizing agent, an aponia-ized agent, a preservative, corrigent, an aromatic, and a coloring agent, can be added and manufactured according to the dosage forms (well-known dosage forms, such as an internal use agent, injections, and a suppository).

[0040] Although an example is given and illustrated about each [these] additive, respectively, it is not limited to especially these. As a solvent, purified water, water for injection, a physiological salt solution, peanut oil, ethanol, a glycerol, etc. can be mentioned. As an excipient, starches, a lactose, grape sugar, white soft sugar, crystalline cellulose, a calcium sulfate, a calcium carbonate, talc, titanium oxide, trehalose, xylitol, etc. can be mentioned. As a coating agent, white soft sugar, gelatin, cellulose acetate phthalate, the macromolecule that carried out [above-mentioned] the publication can be mentioned. As a basis, vaseline, vegetable oil, macro gall, an oil-in-water emulsion nature basis, a water-in-oil emulsion nature basis, etc. can be mentioned. As a binder, synthetic high polymers, such as naturally-ocurring-polymers compounds, such as starch and its derivative, a cellulose and its derivative, gelatin, sodium alginate, tragacanth, and gum arabic, and a polyvinyl pyrrolidone, a dextrin, hydroxypropyl starch, etc. can be mentioned. As lubricant, stearin acid and its salts, talc, waxes, wheat starch, macro gall, hydrogenation vegetable oil, sucrose fatty acid ester, a polyethylene glycol, etc. can be mentioned. As disintegrator, a sodium hydrogencarbonate, a cellulose and its derivative, carmellose calcium, hydroxypropyl starch, a carboxymethyl cellulose, its salts and its bridge formation object, low permutation mold hydroxypropylcellulose, etc. can be mentioned in starch and its derivative, an agar, and the end of gelatin. As a solubilizing agent, cyclodextrin, ethanol, propylene glycol, a polyethylene glycol, etc. can be mentioned. As a suspending agent, gum arabic, tragacanth, sodium alginate, aluminum monostearate, a citric acid, various surfactants, etc. can be mentioned. As a viscous agent, carmellose sodium, a polyvinyl pyrrolidone, methyl cellulose, the hydroxypropyl methylcellulose, polyvinyl alcohol, tragacanth, gum arabic, sodium alginate, etc. can be mentioned. As an emulsifier, gum arabic, cholesterol, tragacanth, methyl cellulose, various

surface active agents, lecithin, etc. can be mentioned. As a stabilizer, a sodium hydrogensulfite, an ascorbic acid, a tocopherol, a chelating agent, inert gas, the reducibility matter, etc. can be mentioned. As a buffer, dibasic sodium phosphate, sodium acetate, a boric acid, etc. can be mentioned. A sodium chloride, grape sugar, etc. can be mentioned as an isotonizing agent. Procaine hydrochloride, lidocaine, benzyl alcohol, etc. can be mentioned as an aponia-ized agent. As a preservative, a benzoic acid and its salts, p-hydroxybenzoic esters, chlorobutanol, invarted soap, benzyl alcohol, a phenol, CHIROMESARU, etc. can be mentioned. As corrigent, white soft sugar, saccharin, glycyrrhiza extract, a sorbitol, xylitol, a glycerol, etc. can be mentioned. Orange peel tincture, rose oil, etc. can be mentioned as an aromatic. As a coloring agent, a water-soluble food color, lake coloring matter, etc. can be mentioned.

[0041] As described above, effectiveness, such as continuation-izing of the effective blood drug concentration of a drug and improvement in bioavailability, is expectable by forming [pharmaceutical preparation / gradual release-ized pharmaceutical preparation, an enteric coated preparation or / drug release control] drugs into DDS pharmaceutical preparation. however, a polypeptide -- in the living body -- deactivation-izing -- or it is decomposed, consequently desired effectiveness may fall or disappear. Therefore, the effectiveness of a component may be made to continuation-ize further by using together the matter which checks deactivation-izing or the matter to disassemble for a polypeptide with the remedy constituent for the therapy of a mesangial cell fecundity nephropathy, and/or prevention. These may be blended into pharmaceutical preparation or may be independently prescribed for the patient. Appropriately, this contractor can identify deactivation-izing or the matter to disassemble for a polypeptide, can choose the matter which checks this, and can blend or use together.

[0042] Into pharmaceutical preparation, the component currently used for the usual constituent as additives other than the above can be used, and the addition of these components can usually be made into an amount in the range which does not bar the effectiveness of this invention.

[0043]

[Example] [Example 1] Well-known approach [Inagi, R.et al.:Biochem.Biophys.Res.Commun., 286:1098-1106, and 2001] It applied correspondingly, human Meg Singh cDNA was rearranged from the culture supernatant of the Chinese hamster ovary cell (CHO) cell which transfected, and HITOMEGUSHIN was obtained. After adding 1M sodium acetate of 100mL(s) to culture supernatant 2L and adjusting to pH4.5, 50mM sodium acetate was added and it diluted twice. Ion exchange chromatography (HiPrep 16/10 SP XL: product made from the Amersham bioscience) was

presented with the diluent (elution conditions: 50mM sodium acetate (pH4.5), NaCl 0-1M linear gradient; elution volume:20x column bed volume). Gel filtration performed buffer exchange (HiPrep 26/10 Desalting, 20mM potassium phosphate (pH6.8)), and hydroxyapatite chromatography (product made from HT-1:Bio-Rad) was continuously presented with the eluate (elution conditions: 20mM potassium phosphate (pH6.8), potassium phosphate 20-400mM linear gradient; elution volume:30x column bed volume). Gel filtration performed buffer exchange again (HiPrep 26/10 Desalting, 50mM MES (pH5.5), 50mM NaCl), and the ion exchange chromatography (Mono S HR 5/5: product made from the Amersham bioscience) was presented with the eluate (elution conditions: 50mM MES (pH5.5), 50mM NaCl, NaCl 50-100mM linear gradient; elution volume:40x column bed volume). Centrifugal thickening of the eluate was carried out using Centricon10 (Millipore make) (3,000g), and buffer exchange was carried out at Dulbecco PBS (-) buffer solution (NISSUI PHARMACEUTICAL make). Centrifugal thickening and buffer exchange were repeated 3 times, and purification recombination Meg Singh was obtained. Chromatography equipment performed all actuation at 4 degrees C using AKTAexplorer 10s (product made from the Amersham bioscience).

[0044] [an example 2] -- MCA assay -- a law -- the polypeptide (TEATAATGSNIVEK) which is equivalent to Meg Singh's P1-P14 array by the method was compounded. The incubation of purification Meg Singh obtained in the above-mentioned example 1 was carried out to the polypeptide which consists of this 14 amino acid for 30 minutes at 37 degrees C by various mole ratios among 0.1M Tris (pH8.0) and 0.05% of Tween20. Then, after making it react for 30 minutes at the plasmin which is a serine protease to Meg Singh, and 37 degrees C, it was made to react with the synthetic fluorescence plasmin substrate of 0.5mM, and Boc(t-butyloxycarbonyl)-Glu-Lys-Lys-MCA (4-methyl-coumaryl-7-amid) (peptide lab), and fluorometry of cutting to AMC from peptide-MCA was performed in lambdaex=380nm and lambdaem=460nm. The polypeptide which consists of 14 amino acid made to arrange at random as contrast was used. A result is shown in drawing 3 . O shows the fluorescence intensity of plasmin. In the case where only Meg Singh is added to plasmin, plasmin activity is checked by Meg Singh and the fluorescence intensity of a plasmin substrate falls (-). Furthermore, when the polypeptide of Meg Singh and this invention was added to plasmin, the polypeptide of this invention checked the inhibition activity over Meg Singh's plasmin on the concentration dependence target, and, as a result, the fluorescence intensity of a plasmin substrate was held (900 times: **, 450 time:**). On the other hand, the inhibition effectiveness was not accepted in the polypeptide of contrast (900 times: **).

[0045]
[Effect of the Invention] The polypeptide which checks the Meg Singh activity was offered by this invention. Moreover, it was checked that the polypeptide which constitutes Meg Singh's reactant loop-formation field can check the Meg Singh activity by this invention. Meg Singh is a molecule constituting amplification of a mesangium substrate, the increment in the cell in a glomerulus, and the self-possessed cause of an immune complex in a glomerulus. These lesions are pathology images characteristic of human mesangial proliferative glomerulonephritis. Amplification of a mesangium substrate is an important lesion in the kidney trouble of many including the nephritis of mesangial proliferation nature. Therefore, the polypeptide which checks Meg Singh's activity is useful in the therapy of the disease accompanied by amplification of a mesangium substrate.
[0046] Moreover, this invention offers the constituent for the therapy of mesangial proliferative glomerulonephritis which contains the polypeptide which checks Meg Singh's activity as an active principle, and/or prevention. As already stated, Meg Singh causes mesangial proliferative glomerulonephritis. Therefore, the polypeptide which checks the Meg Singh activity is useful to the therapy or prevention of mesangial proliferative glomerulonephritis.
[0047]
[Layout Table]
SEQUENCE-LISTING<110> Kurokawa, Kiyoshi Miyata, Toshio<120> Megsin-activity-inhibition factor<130> KRK-A0207<140><141><160> 4<170> PatentIn Ver. 2.1<210> 1<211> 14<212> PRT<213> Artificial Sequence<220> <223> Description of Artificial-Sequence:peptide sequence <400> 1 Thr Glu AlaThr Ala Ala Thr Gly Ser Asn Ile Val Glu Lys 1 5 10 <210> 2 <211> 10<212> PRT <213> Artificial Sequence<220><223> Description of Artificial Sequence:consensus sequence <400> 2 Glu Glu Gly Thr Glu Ala Ala Ala Ala Thr 1 510 <210> 3 <211> 10<212> PRT <213> Artificial Sequence<220><223> Description of Artificial Sequence:peptide sequence <400> 3 Glu Glu Gly Thr Glu Ala Thr AlaAla Thr 1 5 10 <210> 4 <211> 22<212> PRT <213> Artificial Sequence<220><223> Description of Artificial Sequence:peptide sequence <400> 4 Glu Glu Gly Thr Glu Ala Thr Ala Ala Thr Gly Ser Asn Ile Val Glu 1 5 10 15 Lys Gln LeuPro Gln Ser 20

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The mimetic diagram showing the difference in the structure in three kinds of conditions of the reactant loop-formation field which is the active site of a cel pin. (a): An active type, the condition of which (b):cutting was done, (c) : express a **** condition.
[Drawing 2] Drawing showing the protease inhibition mechanism of a cel pin.
[Drawing 3] Drawing showing the result of the MCA assay by the polypeptide of this invention. O : a plasmin and -:plasmin + Meg Singh and **:plasmin + Meg Singh + polypeptide (900 times), a **:plasmin + Meg Singh + polypeptide (450 times), a **:plasmin + Meg Singh + contrast polypeptide (900 times)

[Translation done.]